DEPARTMENT OF THE ARMY
HEADQUARTERS, U.S. ARMY DUGWAY PROVING GROUND
DUGWAY UT 84022-5000

REPLY TO
ATTENTION OF:

FEB 01 2006

CSTE-DTC-DP-WD-LS (70-10p)

MEMORANDUM FOR [redacted] Federal Bureau of Investigation, Washington Field Office, 601 4th Street, NW, Washington, DC 20535

SUBJECT: Final Report for the Analytical Chemistry Analysis of Anthrax Powders, DTC Project Number 8-CO-480-000-0068

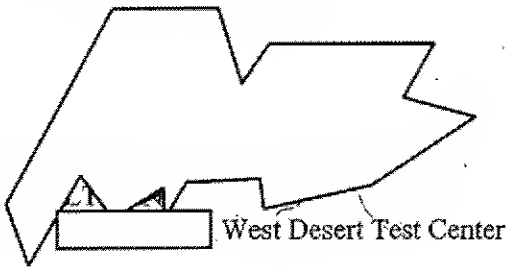
1. Subject report is forwarded to you for retention.

2. Point of contact is Dan Martin, DSN 789-3020, commercial (435) 831-3020.

3. We value your comments; please take a moment to complete our customer survey at http://www.wdtc.army.mil/ServCustomerSurvey.asp

4. West Desert Test Center – "EXCELLENCE IN TESTING."

Encl



DTC Project No. 8-CO-480-000-006
WDTC Document No. WDTC-TR-06-003

FINAL REPORT
FOR THE
ANALYTICAL CHEMISTRY ANALYSIS OF ANTHRAX POWDERS

Principal Author

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WEST DESERT TEST CENTER
U.S. ARMY DUGWAY PROVING GROUND
DUGWAY, UT 84022-5000

JANUARY 2006

Sponsoring Agency:
Federal Bureau of Investigation
Washington Field Office (D. Fetterolf)
601 4th Street, NW
Washington, DC 20535

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BACKGROUND

U.S. Army Dugway Proving Ground (DPG) was asked by the Federal Bureau of Investigation (FBI) to support its investigation into the powdered spores of *Bacillus anthracis* Ames strain found in the letters mailed in the fall of 2001. The Test Execution Directive from U.S. Army Developmental Test Command directed DPG to conduct the proposed project (Reference 1).

NOTE: The format of this report is as per the customer's request.

MATERIALS AND METHODS

Stock Culture

Bacillus anthracis Ames strain from the original letter sent to Senator Leahy, as received from the FBI couriers, was used as the starter source for all propagated material in this work. Upon receipt of the sample, the organism was cultured directly from the powder for colony isolation on Tryptic Soy Agar (TSA). Three isolated colonies were selected and resuspended in cold, sterile, water for irrigation (WFI). This suspension was used to inoculate four blood agar plates for confluent growth that were incubated at 37°C for 48 hours. After the cultures had sporulated, the spores were harvested into sterile WFI. An aliquot was reserved and the rest were heat shocked for 25 minutes at 61°C to kill all vegetative cells. Samples from before and after heat shocking were assayed by plate count on TSA. The heat shocked suspension was then divided into 15 aliquots and stored at –60°C. This material was used as stock inoculum for all agar and fermentor production runs pertaining to this project.

Production on Agar Plates

Stock spore inoculum was thawed and diluted in approximately 40 mL of sterile WFI from one of the frozen aliquots and vortex mixed. A 0.25 mL volume of this suspension was spread onto either a 150 mm sheep blood agar (SBA) plate or new sporulation medium (NSM) plate (for confluent growth) and incubated at 37±2°C for 3 to 9 days. After sporulation, the paste material on the agar surface was harvested from the plates and placed in sterile, 90-mL tubes and stored until further processed. This growth process was repeated until there was sufficient paste material to meet the requirements.

Washing of the Paste

Paste material was weighed and then resuspended in a ratio of 1:10 (mass:volume) of paste to cold, sterile WFI (designated as the Dugway wash method) or cold, sterile phosphate buffered saline (PBS) with 0.2% Alcojet® [(Alconox, Inc., White Plains, New York) as part of the Patrick wash method to be described]. The material resuspended in the PBS/Alcojet® was also homogenized in a glass Waring® (Torrington, Connecticut) blender and centrifuged in new sterile, gasketed polypropylene 500-mL centrifuge bottles at approximately 7000 RPM. The supernatant was decanted and any loose cell debris or vegetative cells on the surface of the pellet removed by swirling some of the supernatant over the surface. The final pellet was weighed. This repre-

sented one wash cycle. This was repeated two additional times. Samples (small aliquots of the paste) were archived in a frozen state at the end of each step. After washing, all material was stored at 4°C until further processed. After several weeks of storage at 4°C, the decision was made by the customer to store at –65°C until the material was further processed.

Production by Fermentation

Two 500-mL volumes of 2X nutrient broth were inoculated with 0.05 mL of the seed stock material and incubated overnight at 34°C. Three milliliters of Sigma® (St. Louis, Missouri) Antifoam 204 (without silica) were added to 17.2 L of 2X nutrient broth and then sterilized in a BioFlo 4500 fermentor (New Brunswick Scientific Co., Inc., Edison, New Jersey) for 45 minutes. On the following day, 4.3 L of Leighton-Doi salts/sugar additives were introduced into the fermentor along with the two 500-mL, 18-hour inocula. The culture was grown at 34°C, with an air sparger output of 20 standard liters per minute flow, and 400 RPM agitation for approximately 48 hours. The culture material was then centrifuged in 1-L clean, sterile, gasketed centrifuge bottles at approximately 6000 RPM and the paste material was stored at 4°C until further processed (Lot 05AUG05). This same process was repeated on a second batch with the substitution of Sigma Antifoam C emulsion instead of Antifoam 204 in a total volume of approximately 20 L (Lot 01SEP05).

Drying

Oven Dried – The oven-drying procedures proceeded as follows: A drying oven was placed within a glove box. The portion of paste to be oven dried was placed in a clean, sterile agate mortar, then into the drying oven. The NSM agar material was dried for 66 hours at 40°C under vacuum. The SBA material was dried for 72 hours with 40°C as the target temperature. The actual temperature spiked at the end of the drying time to 48°C for an unknown length of time.

Freeze Dried – The freeze-drying procedure proceeded as follows: The portion to be lyophilized was resuspended in cold, sterile WFI with the spore concentration. The material was then dispensed into sterile glass lyophilization containers and shell frozen. The containers with the frozen material were placed under lyophilization conditions (using a Duradry µp by FTS Systems, Stone Ridge, New York) until suitable dryness was achieved. Teflon® stoppers were placed so as to allow sealing of the vials prior to releasing the vacuum.

Speed Vac Dried – All samples to be dried by the Speed Vac method were resuspended in cold, sterile, WFI at approximately 0.1 g/mL. These were transferred into several sterile polypropylene tubes and frozen at –70°C for 24 hours. The samples were then placed in the machine [Savant (Ramsey, Minnesota) Speed Vac, Model SPD1010-115] and dried for 24 hours, after which they were ready for further processing.

Acetone Dried – Some residnal paste harvested from SBA and NSM media and washed using both methods was set aside for acetone drying. The masses of paste used were 1.5 g of SBA/Patrick, 1.6 g of NSM/Patrick, 1.3 g of SBA/Dugway, and 1.3 g of NSM/Dugway. After completing the procedure, the materials were placed in sterile agate mortars within a glove box. Half were allowed to finish drying and half were allowed to dry for 7 days. The additional dry-

ing was for the purpose of allowing the acetone to more fully evaporate. The humidity and temperature in the glove box were monitored.

Milling

Three methods of milling, as described below, were performed with designated portions of the oven dried, freeze dried, and Speed Vac dried materials.

Sieve – Several milling sieve apparatuses were constructed for the sieve process. Stainless steel wire mesh (40, 60, 80, and 100 mesh) integrated into rubber gaskets were affixed between stainless steel, airtight containers measuring 4.5 cm in diameter and each containing 3 to 5 sterile, stainless steel ball bearings, approximately 1 cm in diameter (see Figures C.21 and C.22). An air piston was affixed to the outside of the assembled fixture and attached to an air line. When operated, the piston caused the ball bearings to vibrate and force the dried material through the sieves. The final product was collected from the lower container and placed within a numbered, preweighed, clean, sterile I-Chem™ Brand vial (Chase Scientific Inc., Rockwood, Tennessee) until divided into aliquots.

Ball Mill – Into each of several 1-pint sized ceramic milling jars were placed approximately 25 sterile, zirconium, milling cylinders measuring 1.2 × 1.3 cm (see Figure C.20). Each sample to be milled was then placed within the gasketed, airtight jar and rotated for approximately 16 hours (overnight) on a fixed platform within a glove box or within a biosafety cabinet. The powder was then extracted from the jar by using sterile, plastic cell scrapers and placed within a numbered, pre-weighed, clean, sterile I-Chem™ vial until divided into aliquots.

Mortar and Pestle – All dried material to be milled by mortar and pestle were placed in the mortar within a glove box and ground with a pestle until a fine powder resulted (see Figures C.15 and C.16). All powdered material was then placed within a numbered, preweighed, clean, sterile I-Chem™ vial until divided into aliquots.

Sample Division and Irradiation

After all 36 preparations were dried and milled, each was measured to determine the total mass. Each was then divided into ten sterile, polypropylene cryotubes labeled a through j and with the appropriate identifier such as NPVB (New sporulation medium, Patrick wash method, Speed Vac drying, and Ball milled) to describe how each one was processed. The codes used throughout the remainder of this report are shown in Table 1.

Aliquots f through j were selected for gamma irradiation in order to ship them to designated laboratories. The materials were irradiated at the facility operated by the Life Sciences Division at DPG using a ^{60}Cobalt source. The usual exposure was approximately 4×10^6 RADs or 40 kGy.

Table 1. Sample Codes.

Media	N = New sporulation medium S = Sheep blood agar
Wash Methods	D = Dugway wash method (no detergent or blending) P = Patrick wash method (with detergent and a blending step)
Drying Methods	O = Oven dried L = Lyophilized V = Speed Vac dried
Milling Methods	B = Ball mill method M = Mortar and pestle method I = Sieve method

Sterility Determination

Sterility verification was performed as follows: Approximately 10% from each sample to be confirmed sterile was aseptically removed and placed in sterile growth medium in a volume appropriate to the amount of sample (e.g., 0.1 g into 10 mL broth). The broth was then incubated for 72 hours, along with positive and negative controls. After this incubation, an inoculation loopfull (or up to 1 mL) of the broth was transferred from each tube (or flask), including positive and negative controls, to an agar plate and incubated for 48 hours and observed for growth. The results were observed and recorded.

Sample Assay

An aliquot from each of the 36 preparations was utilized to determine the number of colony forming units (CFU) per gram of material. A measured portion of each was weighed and then resuspended with vortex mixing in 10 mL of sterile PBS with 0.2% Alcojet® detergent and approximately 50 µL of Sigma® 289 Antifoam. The suspension was then mixed for 1 minute with a Cole Parmer (Vernon Hills, Illinois) hand-held homogenizer using a sterile, disposable tip for each preparation. Serial dilutions were then made and several dilutions assayed in triplicate by standard plate count method on TSA plates.

Electron Microscopy

An aliquot from each preparation was selected, and electron microscope stubs were prepared with adhesive, black surfaces and placed in holders within clean, plastic tubes coated with an antistatic coating. A very small amount from each aliquot was removed and placed in a separate plastic tube, each having its own electron microscope stub. Each tube was then shaken to aerosolize the powder and then placed so as to allow the aerosolized particles to settle on the electron microscope stub. All tubes were allowed to remain motionless overnight.

All stubs were subsequently opened and placed within a sealable tray with one vial of osmium tetroxide (OsO_4) crystals. The tray was sealed and placed within a black plastic bag and left in a biological safety cabinet for 24 hours. Two glass microscope slides (each having a similar amount of preparation NPVI as that on the stubs) were also prepared as controls. One had been placed within the tray for exposure to the OsO_4 and the other kept in a separate sterile tube. Upon demonstration of sterility of the control slide, each sample was then examined by scanning electron microscopy.

Elemental Analysis

In viewing the electron microscope pictures, crystals were noted in some of the preparations. This prompted a request that some elemental analysis be performed using a new addition to the electron microscope to determine what the crystals might be. The analytical method used was Energy Dispersive Spectroscopy (EDS) (Reference 2) using a Princeton Gamma Tech EDS instrument (Princeton, New Jersey). The samples that were previously prepared for electron microscopy were used for this analysis. An osmium standard was also analyzed to verify its peak location and account for any background to resolve any coinciding peaks (e.g., phosphorus).

Particle Sizing

An aliquot from the final dried preparations was used for particle sizing. A full aliquot was required in order to perform the sizing. All manipulations with the powdered material occurred within a filtered glove box. An individual aliquot was placed in the sample holder of a TSI® Inc. (St. Paul, Minnesota) Model 3225 Aerosizer DSP. The sample was aerosolized by means of a TSI® Aerodisperser Model 3230, and the aerosolized powder was directed through the measuring channel. The sample holder was cleaned and the conduit to the measuring chamber air washed thoroughly between measurements. A calibrated standard particle of 7 μm was run for calibration purposes at intervals throughout the measuring session.

Cell Count Versus Colony-Forming-Unit Count

A portion of one of the aliquots of final dried material was resuspended in cold, sterile WFI and assayed for CFU per milliliter as described in the Sample Assay section above. The same sample (or a dilution thereof) was also observed in an Improved Neubauer Hemacytometer (Horsham, Pennsylvania) and a particle count per milliliter was determined according to the manufacturer's directions.

Analysis of Data

The CFU per gram, percent viable particles per gram, and particle size data were analyzed using analysis of variance (ANOVA) with a 95% level of confidence to determine the effects of the various methods of growth, washing, drying, and milling to yield the final products.

Although there was just one sample per complete set of conditions (medium, wash method, drying method, and milling method), the analysis could be performed because there were multiple variables for which a control mechanism could be used. Consider the medium variable: an

attempt could be made to distinguish between medium A and medium B because there was an observation for each of these media at every possible wash/dry/mill variable combination. Numerically, these combinations provide 18 (2 wash × 3 drying × 3 milling methods) paired medium observations.

Photo Documentation

Appendix C contains Figures C.1 to C.22. These figures document several of the steps of this project in terms of the preparation of the materials produced.

RESULTS

Stock Material

The CFU per milliliter count on the stock material prepared from the agar plates and used for all plate inoculations was 2.8×10^7 CFU/mL for the pre-heat-shocked material and 9.65×10^7 CFU/mL for the after-heat-shocked material. Such an increase may be an indication that the heat shock caused some chains having viable spores within to be released as individual spores thereby increasing the CFU count per milliliter.

Harvests of Agar Plate Production Runs

The first set of SBA plates were incubated for 5 days prior to harvest. Ninety-nine plates were harvested and a total of 38.6 g were placed in storage at 4°C. Table 2 gives an example of a typical harvest from agar plates.

Table 2. Typical Harvest From Agar Plates.

Agar Type[a]	Hours Incubated	Lot Number	Number of Plates Harvested	Amount of Paste Recovered (g)
SBA	120	07JAN03	99	38.6
NSM[b]	72	—	256+	92

[a]SBA – sheep blood agar; NSM – new sporulation medium.
[b]This agar was prepared in-house at the Life Sciences Division.

Fermentation Product

Thirteen and one half grams of the first fermentation product were provided as irradiated material. Thirty-one grams of paste from Lot 05AUG05 was initially irradiated, and subsequently, the remaining 28 g were irradiated again. From Lot 01SEP05, 20.5 g of paste were irradiated.

After confirming sterility, a certificate of sterility was created for each and the remainder of the inactivated material was shipped to the customer or designated recipient.

Any other irradiation of materials was performed in the same manner.

Speed Vac Drying Procedure

During the drying run with the Speed Vac, there was a power failure during the night and the samples all thawed. They were refrozen and run again. Another malfunction caused the samples to thaw overnight. They were refrozen and run again. The third attempt was successful and the samples were fully dry on the following morning.

Sample Division and Irradiation

Table 3 shows the mass for each final product at the conclusion of all growth, washing, drying, and milling processes. At this point it was decided that 10 aliquots of each would be dispensed at approximately 25 mg each. For samples that did not have sufficient mass for a 25-mg sample, smaller aliquots were made (e.g., SPVB). Five of these would remain as viable material while five were to be gamma irradiated for total inactivation. Each aliquot was placed within a sterile, 1-mL polypropylene cryotube.

Irradiation of Aliquots

The irradiation of materials was performed at DPG using an MDS Nordion (Ottawa, Ontario, Canada) Gamma Cell model Excel 200 irradiator. Table 4 shows the amount of irradiation given to each sample along with the result of the sterility check and other information. If the results of the sterility check after irradiation showed no viable organisms in the samples tested, then a certificate of sterility was prepared and provided with the materials shipped (see Appendix A).

Electron Microscopy

Samples of the dried/milled materials were placed on adhesive electron microscopy stubs then exposed to OsO_4, along with an extra sample to serve as a test sample for sterility testing. Upon release as biologically inactive, the samples were examined by electron microscopy with a JEOL (Peabody, Massachusetts) JSM-6300F scanning electron microscope.

Particle Sizing

One aliquot each of the dried/milled samples was used for particle sizing. Each was sized using the Aerosizer™ particle sizer (Malvern Instruments, Worcestershire, United Kingdom) within a glove box under biosafety, level 3 (BL3) conditions. Table 5 shows the mean diameter of three readings with a grand mean for each sample.

Table 3. Total Amount (in Grams) of Final Dry Preparation Produced for Each of the 36 Samples.

NDOB	NPOB	SDOB	SPOB	NDLB	NPLB	SDLB	SPLB	NDVB	NPVB	SDVB	SPVB
0.772	0.635	0.736	0.537	1.164	0.578	0.789	0.763	0.259	0.484	0.294	0.077
NDOI	**NPOI**	**SDOI**	**SPOI**	**NDLI**	**NPLI**	**SDLI**	**SPLI**	**NDVI**	**NPVI**	**SDVI**	**SPVI**
0.458	0.738	0.513	0.442	1.665	1.282	0.879	0.652	0.282	0.327	0.374	0.271
NDOM	**NPOM**	**SDOM**	**SPOM**	**NDLM**	**NPLM**	**SDLM**	**SPLM**	**NDVM**	**NPVM**	**SDVM**	**SPVM**
1.412	0.941	1.025	0.516	1.681	1.755	0.747	1.195	0.353	0.712	0.781	0.668

1) **S** = Sheep blood agar, **N** = New sporulation medium, **D** = Dugway wash method, **P** = Patrick wash method, **O** = Oven dried, **V** = Speed Vac dried, **L** = Lyophilized, **B** = Ball milled, **I** = Steve milled, **M** = Mortar and pestle.
2) Red = ball mill, green = sieve, blue = mortar and pestle.

Table 4. Irradiated Materials.

Sample Description	Date Irradiated and DTC[a] Number	Amount of Irradiation and Time Interval	Result of Sterility Check	Disposition
Aliquots f to j of the 36 dried preparations	21 OCT 2003 DTC 0101	35.59 kGy over 125 minutes	Test sample and negative control showed no growth. Positive control showed growth	Death certificate made and samples stored at room temperature pending use or shipment.
13.5 g of paste from Lot 20MAY04	19 AUG 2004 DTC 0121	34.56 kGy	Test sample and negative control showed no growth. Positive control showed growth.	Death certificate made. Sample stored at 4°C prior to shipment.
31 g of paste from Lot 05AUG05	08 AUG 2005	39.41 kGy over 148 minutes	**Test sample and positive control showed growth**. Negative control showed no growth.	**To be irradiated again**.
Remaining 28 g of irradiated Lot 05AUG05	18 AUG 2005 DTC 0210	41.37 kGy over 155 minutes	Test sample and negative control showed no growth. Positive control showed growth.	Death certificate made. Sample stored at 4°C prior to shipment.
20.5 g of Lot 01SEP05	06 SEP 2005 DTC 0211	44.29 kGy over 160 minutes	Test sample and negative control showed no growth. Positive control showed growth.	Death certificate made. Sample stored at 4°C prior to shipment.
Material received from NMRC[b]	12 SEP 2005 DTC 0212	50.07 kGy over 160 minutes	Test sample and negative control showed no growth. Positive control showed growth.	Death certificate made. Sample stored at 4°C prior to shipment.

[a]Death certificate.
[b]Naval Medical Research Center.

Table 5. Particle Sizing Data From Each of the 36 Sample Dried/Milled *Bacillus anthracis* Preparations.

Calibration	Sample ID	DONE	Mean Diameter 1	2	3	Grand Mean	Calibration	Sample ID	DONE	Mean Diameter 1	2	3	Grand Mean
	Stds 7 µm*	10/20/04	7.581	7.531		7.556							
	Stds 7 µm*	10/21/04	7.633	7.624	7.682	7.646							
1	SPLB	10/21/04	9.280	7.231	7.034	7.848	19	NPLB	10/22/04	5.388	5.099	4.627	5.038
2	SPLI	10/21/04	2.301	2.223	2.304	2.276	20	NPLI	10/22/04	2.317	2.257	2.344	2.306
3	SPLM	10/21/04	2.081	2.107	1.984	2.057	21	NPLM	10/22/04	2.283	2.205	2.215	2.234
4	SPOB	10/21/04	6.886	7.004	7.378	7.089	22	NPOB	10/22/04	3.603	3.567	3.427	3.532
5	SPOI	10/21/04	5.915	4.976	7.071	5.987	23	NPOI	10/22/04	4.126	4.221	4.579	4.309
6	SPOM	10/21/04	8.120	8.487	7.403	8.003	24	NPOM	10/22/04	4.654	4.389	4.572	4.538
	Stds 7µm*	10/21/04	7.608	7.619	7.598	7.608		CAL 7µm*	10/25/04	7.536	7.514	7.528	7.526
START	CAL 7µm*	10/22/04	7.654	7.637	7.645	7.645		CAL 7µm*	10/25/04	7.641	7.656	7.615	7.637
7	SPVB	10/22/04	9.884	9.889	10.37	10.05	25	NPVB	10/25/04	7.029	6.147	5.740	6.305
8	SPVI	10/22/04	4.831	11.95	7.847	8.209	26	NPVI	10/25/04	2.285	2.199	2.330	2.271
9	SPVM	10/22/04	2.799	2.916	1.626	2.447	27	NPVM	10/25/04	2.257	2.407	2.266	2.310
10	SDLB	10/22/04	2.131	2.219	2.107	2.152	28	NDLB	10/25/04	3.359	4.012	4.235	3.869
11	SDLI	10/22/04	2.270	2.084	1.974	2.109	29	NDLI	10/25/04	2.612	2.459	2.519	2.530
12	SDLM	10/22/04	2.033	2.233	2.076	2.114	30	NDLM	10/25/04	2.703	2.799	2.655	2.719
	CAL 7µm*	10/22/04		5.765	6.449	6.107		CAL 7µm*	10/25/04	7.632	7.654	7.275	7.520
13	SDOB	10/22/04	7.269	7.846	7.473	7.529	31	NDOB	10/25/04	11.71	11.49	9.554	10.92
14	SDOI	10/22/04	7.037	6.637	12.35	8.675	32	NDOI	10/25/04	13.93	6.473	13.98	11.46
15	SDOM	10/22/04	7.034	6.652	5.325	6.337	33	NDOM	10/25/04	3.561	12.150	12.940	9.550
16	SDVB	10/22/04	7.005	6.671	6.684	6.787	34	NDVB	10/25/04	5.353	4.753	4.397	4.834
17	SDVI	10/22/04	3.439		3.023	3.231	35	NDVI	10/25/04	2.821	2.549	2.475	2.615
18	SDVM	10/22/04	3.254	3.529	4.374	3.719	36	NDVM	10/25/04	2.510	2.522	2.799	2.610
	CAL 7µm*	10/22/04	7.017	7.578	7.600	7.398		CAL 7µm*	10/25/04	4.842	6.577	7.515	6.311
								CAL 7µm*	10/25/04	7.019	7.529	7.260	7.269

1) **S** = Sheep blood agar, **N** = New sporulation medium, **D** = Dugway wash method, **P** = Patrick wash method, **O** = Oven dried, **V** = Speed Vac dried, **L** = Lyophilized, **B** = Ball milled, **I** = Sieve milled, **M** = Mortar and pestle.
2) Red = ball mill, green = sieve, blue = mortar and pestle, * = Polystyrene microspheres.

Acetone Dried Material

From the irradiated, acetone-dried NSM/Patrick material, five aliquots from the 83 mg were transferred into I-Chem™ vials for shipment to the FBI. The aliquots measured 10 mg, 10 mg, 10 mg, 11 mg, and 12 mg. The remainder (approximately 30 mg) was stored at 4°C. The data from monitoring the temperature and humidity during the extended drying times are shown in Figures 1 and 2.

Plate Count of the Dried Preparations

The counts from the various dried preparations varied considerably as seen in Table 6. The lowest concentration was 8.47×10^6 CFU/g for NDLB and the highest was 2.11×10^{11} CFU/g for NDOM. The ball-milled materials generally had the lowest concentrations with a high of 1.82×10^9 CFU/g, while all other preparations were generally in the range of 10^{10} to 10^{11} CFU/g.

Cell Count Versus CFU Count

The results from the comparison of physical cell counts and CFUs per milliliter are found in Table 7. The percent viable CFU in the particles ranged from less than 0.01% for NDLI to 39.29% for NDOM. The values for all samples from the ball-milling are shown with "Clumping" and "No data." There was excessive clumping of particles together in these samples that precluded getting a reasonable determination of how many particles (spores) per milliliter were in the sample.

Elemental Analysis

After the scans were performed, the elements reported to have been present in the area of the crystal (including the crystal) were carbon, oxygen, nitrogen, sodium, magnesium, aluminum, silicon, osmium, phosphorus, sulfur, calcium and antimony.

The antimony and the calcium peaks were superimposed. There was no calcium standard available to attempt subtracting a background, however, according to the image, the antimony and calcium were detected in the same area (the crystal).

The spectrum for osmium and phosphorus appeared in the same peak. However, when an osmium standard was run and identified, the auto identification recognized the osmium peak as osmium and phosphorus even though, according to the manufacturer of the osmium standard (Electron Microscopy Sciences, Hatfield, Pennsylvania), phosphorus was not present. Phosphorus and osmium x-ray energies are very close to one another, as detected by this analytical method.

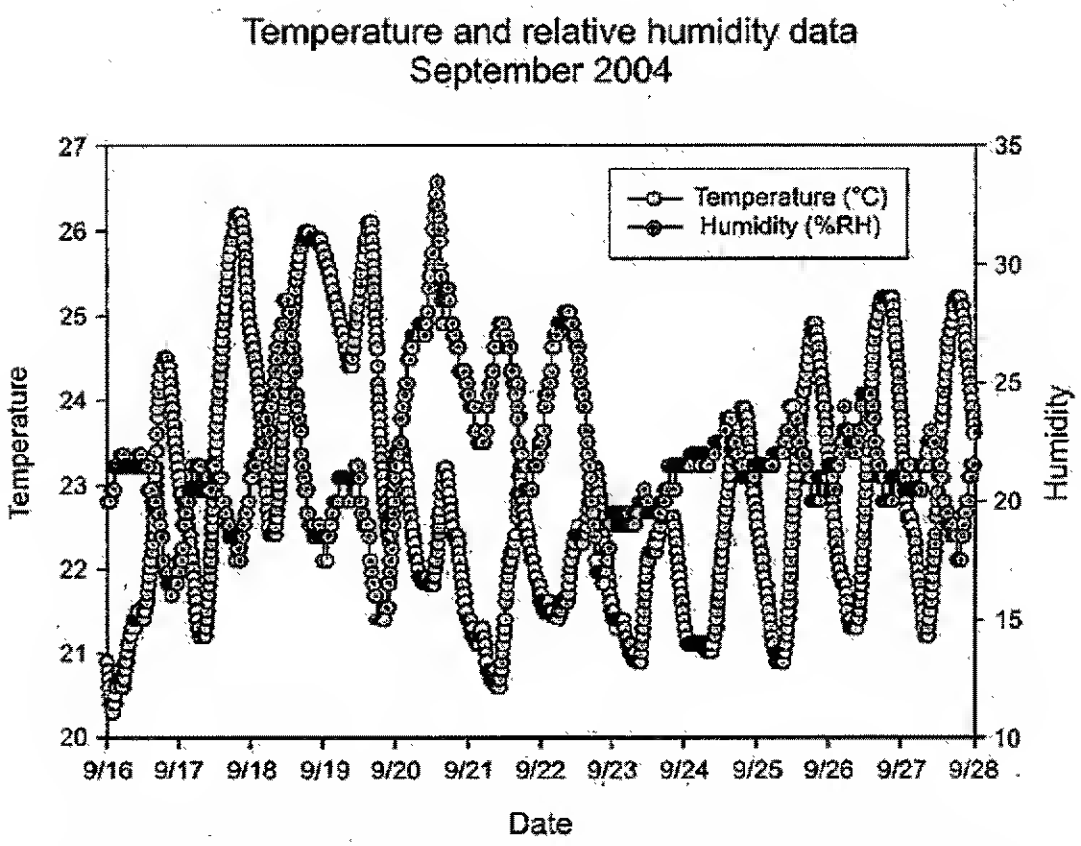
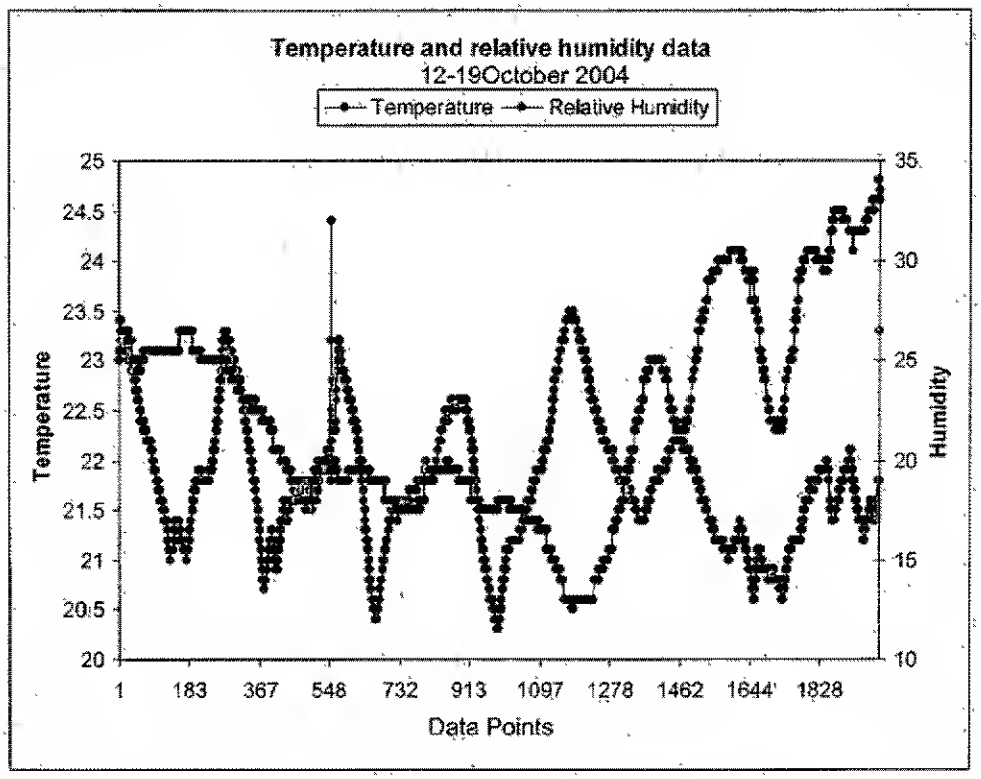


Figure 1. Temperature and Relative Humidity Data From Drying Periods – September 2004.



NOTE: The spike at data point 548 and at the end is where the data logger was unplugged momentarily.

Figure 2. Temperature and Relative Humidity Data From Drying Periods – October 2004.

Table 6. Colony Forming Units (CFU) per Gram Results From Plate Count Assay of Each of the 36 Dry Preparations.

Sample ID	Counts				Amount Plated	Total Volume	Dilution	Grams in Suspension	CFU/g
	Plate1	Plate2	Plate3	AVG					
SDLB	2	2	2	2	0.2 mL	10 mL	1.E-05	0.0614	1.63E+08
SDOB	73	71	67	70	0.2 mL	10 mL	1.E-03	0.0459	7.63E+07
SDVB	238	241	214	231	0.2 mL	10 mL	1.E-03	0.0344	3.36E+08
SPLB	43	69	61	58	0.2 mL	10 mL	1.E-03	0.0534	5.40E+07
SPOB	242	225	189	219	0.2 mL	10 mL	1.E-03	0.0481	2.27E+08
SPVB	74	66	91	77	0.2 mL	10 mL	1.E-03	0.0322	1.20E+08
NDLB	**10**	**11**	**14**	**12**	**0.2 mL**	**10 mL**	**1.E-03**	**0.0689**	**8.47E+06**
NDOB	**77**	**101**	**87**	**88**	**0.2 mL**	**10 mL**	**1.E-03**	**0.0461**	**9.94E+07**
NDVB	**9**	**13**	**23**	**15**	**0.2 mL**	**10 mL**	**1.E-05**	**0.0411**	**1.82E+09**
NPLB	**133**	**111**	**98**	**114**	**0.2 mL**	**10 mL**	**1.E-04**	**0.041**	**1.39E+09**
NPOB	**231**	**239**	**203**	**224**	**0.2 mL**	**10 mL**	**1.E-03**	**0.0381**	**2.94E+08**
NPVB	**44**	**61**	**56**	**54**	**0.2 mL**	**10 mL**	**1.E-04**	**0.0423**	**6.34E+08**
SDLI	62	62	61	62	0.2 mL	10 mL	1.E-06	0.0569	5.42E+10
SDOI	62	70	66	66	0.2 mL	10 mL	1.E-06	0.0618	5.34E+10
SDVI	66	75	82	74	0.2 mL	10 mL	1.E-06	0.0459	8.10E+10
SPLI	62	59	61	61	0.2 mL	10 mL	1.E-06	0.0346	8.77E+10
SPOI	83	118	122	108	0.2 mL	10 mL	1.E-06	0.0366	1.47E+11
SPVI	43	28	37	36	0.2 mL	10 mL	1.E-06	0.0255	7.06E+10
NDLI	**40**	**41**	**30**	**37**	**0.2 mL**	**10 mL**	**1.E-05**	**0.0385**	**4.81E+09**
NDOI	**117**	**113**	**114**	**115**	**0.2 mL**	**10 mL**	**1.E-06**	**0.039**	**1.47E+11**
NDVI	**132**	**117**	**120**	**123**	**0.2 mL**	**10 mL**	**1.E-06**	**0.0563**	**1.09E+11**
NPLI	**73**	**54**	**64**	**64**	**0.2 mL**	**10 mL**	**1.E-06**	**0.0344**	**9.25E+10**
NPOI	**120**	**120**	**123**	**115**	**0.2 mL**	**10 mL**	**1.E-06**	**0.0555**	**1.03E+11**
NPVI	**98**	**114**	**111**	**108**	**0.2 mL**	**10 mL**	**1.E-06**	**0.0456**	**1.18E+11**

Table 6. Colony Forming Units (CFU) per Gram Results From Plate Count Assay of Each of the 36 Dry Preparations (Cont'd).

Sample ID	Counts				Amount Plated	Total Volume	Dilution	Grams in Suspension	CFU/g
	Plate 1	Plate 2	Plate 3	AVG					
SDOI Vortex only	79	93	78	83	0.2 mL	10 mL	1.E-06	0.0618	6.74E+10
SDLM	63	71	52	62	0.2 mL	10 mL	1.E-06	0.0646	4.80E+10
SDOM	68	87	82	79	0.2 mL	10 mL	1.E-06	0.0611	6.46E+10
SDVM	65	57	68	63	0.2 mL	10 mL	1.E-06	0.0583	5.43E+10
SPLM	23	30	46	33	0.2 mL	10 mL	1.E-06	0.026	6.35E+10
SPOM	68	76	71	72	0.2 mL	10 mL	1.E-06	0.0575	6.23E+10
SPVM	242	272	251	255	0.2 mL	10 mL	1.E-05	0.0229	5.57E+10
NDLM	**72**	**68**	**71**	**70**	**0.2 mL**	**10 mL**	**1.E-05**	**0.0367**	**9.58E+09**
NDOM	**152**	**145**	**129**	**142**	**0.2 mL**	**10 mL**	**1.E-06**	**0.0336**	**2.11E+11**
NDVM	**161**	**169**	**156**	**162**	**0.2 mL**	**10 mL**	**1.E-06**	**0.0401**	**2.02E+11**
NPLM	**69**	**72**	**81**	**74**	**0.2 mL**	**10 mL**	**1.E-06**	**0.0589**	**6.28E+10**
NPOM	**63**	**52**	**68**	**61**	**0.2 mL**	**10 mL**	**1.E-06**	**0.0279**	**1.09E+11**
NPVM	**87**	**97**	**89**	**91**	**0.2 mL**	**10 mL**	**1.E-06**	**0.0457**	**9.96E+10**
SPLM Vortex only	158	184	179	174	0.2 mL	10 mL	1.E-05	0.026	3.34E+10

1) S = Sheep blood agar, N = New sporulation medium, **D** = Dugway wash method, **P** = Patrick wash method, **O** = Oven dried, **V** = Speed Vac dried, **L** = Lyophilized, **B** = Ball milled, **I** = Sieve milled, **M** = Mortar and pestle.
2) **Red** = ball mill, **green** = sieve, **blue** = mortar and pestle.
3) ID = Identification, AVG = Average, CFU = Colony forming unit.

Table 7. Physical Cell Counts With Improved Neubauer Hemacytometer and Colony Forming Units (CFU) per Milliliter Counts on Suspensions.

Sample ID	Average of 16 Squares, i.e., = 0.04 mm^2	Times 25 = 1 mm^2	Dilution Factor (From Resus-pension)	Mult. Factor to Reflect 1 mL	Cells/mL	CFU Plate Counts			AVG	Dilution Factor	Mult. Factor for Vol.	CFU/mL	Percent Viable Spores
NPLB	CLUMPING*	No data		10000	No data	231	272	254	252	1.0E+04	5	1.E+07	No data
NPLI	185	4625	20	10000	9.E+08	69	69	77	72	1.0E+06	5	4.E+08	38.74
NPLM	180	4500	50	10000	2.E+09	115	124	143	127	1.0E+06	5	6.E+08	28.30
NPOB	CLUMPING*	No data		10000	No data	82	68	71	74	1.0E+04	5	4.E+06	No data
NPOI	183	4575	200	10000	9.E+09	60	68	54	61	1.0E+07	5	3.E+09	33.15
NPOM	376	9400	200	10000	2.E+10	98	87	90	92	1.0E+07	5	5.E+09	24.38
NPVB	CLUMPING*	No data		10000	No data	146	148	150	148	1.0E+04	5	7.E+06	No data
NPVI	271	6775	50	10000	3.E+09	126	142	115	128	1.0E+06	5	6.E+08	18.84
NPVM	194	4850	400	10000	2.E+10	126	140	121	129	1.0E+07	5	6.E+09	33.25
NDLB	CLUMPING*	No data		10000	No data	83	64	71	73	1.0E+05	5	4.E+07	No data
NDLI	520	2600	400	10000	1.E+10	37	47	42	42	1.0E+03	5	2.E+05	0.00
NDLM	214	5350	100	10000	5.E+09	111	124	128	121	1.0E+05	5	6.E+07	1.13
NDOB	CLUMPING*	No data		10000	No data	110	91	129	110	1.0E+04	5	6.E+06	No data
NDOI	159	3975	400	10000	2.E+10	121	97	83	100	1.0E+07	5	5.E+09	31.55
NDOM	168	4200	400	10000	2.E+10	126	120	150	132	1.0E+07	5	7.E+09	39.29
NDVB	CLUMPING*	No data		10000	No data	32	32	41	35	1.0E+05	5	2.E+07	No data
NDVI	158	3950	200	10000	8.E+09	65	46	59	57	1.0E+07	5	3.E+09	35.86
NDVM	228	5700	400	10000	2.E+10	170	155	161	162	1.0E+07	5	8.E+09	35.53

Table 7. Physical Cell Counts With Improved Neubauer Hemacytometer and Colony Forming Units (CFU) per Milliliter Counts on Suspensions (Cont'd).

Sample ID	Average of 16 Squares, i.e., = 0.04 mm^2	Times 25 = 1 mm^2	Dilution Factor (From Resus-pension)	Mult. Factor to Reflect 1 mL	Cells/mL	CFU Plate Counts			AVG	Dilution Factor	Mult. Factor for Vol.	CFU/mL	Percent Viable Spores
SPLB	CLUMPING*	No data		10000	No data	99	90	95	95	1.0E+03	5	5.E+05	No data
SPLI	145	3625	100	10000	4.E+09	191	214	232	212	1.0E+06	5	1.E+09	29.29
SPLM	141	3525	100	10000	4.E+09	132	138	135	135	1.0E+06	5	7.E+08	19.15
SPOB	CLUMPING*	No data		10000	No data	136	113	142	130	1.0E+06	5	7.E+08	No data
SPOI	366	9150	100	10000	9.E+09	48	41	54	48	1.0E+07	5	2.E+09	26.05
SPOM	265	6625	100	10000	7.E+09	21	30	32	28	1.0E+07	5	1.E+09	20.88
SPVB	CLUMPING*	No data		10000	No data	46	70	79	65	1.0E+04	5	3.E+06	No data
SPVI	69	1725	100	10000	2.E+09	68	72	84	75	1.0E+06	5	4.E+08	21.64
SPVM	203	5075	100	10000	5.E+09	117	138	123	126	1.0E+06	5	6.E+08	12.41
SDLB	CLUMPING*	No data		10000	No data	23	34	22	26	1.0E+04	5	1.E+06	No data
SDLI	130	3250	100	10000	3.E+09	122	105	95	107	1.0E+06	5	5.E+08	16.51
SDLM	302	7550	100	10000	8.E+09	162	148	166	159	1.0E+06	5	8.E+08	10.51
SDOB	CLUMPING*	No data		10000	No data	150	135	130	138	1.0E+03	5	7.E+05	No data
SDOI	444	11100	100	10000	1.E+10	47	56	45	49	1.0E+07	5	2.E+09	22.22
SDOM	544	13600	100	10000	1.E+10	63	49	64	59	1.0E+07	5	3.E+09	21.57
SDVB	CLUMPING*	No data		10000	No data	70	81	75	75	1.0E+04	5	4.E+06	No data
SDVI	469	11725	100	10000	1.E+10	53	46	42	47	1.0E+07	5	2.E+09	20.04
SDVM	143	3575	100	10000	4.E+09	61	69	68	66	1.0E+06	5	3.E+08	9.23

1) **S** = Sheep blood agar, **N** = New sporulation medium, **D** = Dugway wash method, **P** = Patrick wash method, **O** = Oven dried, **V** = Speed Vac dried, **L** = Lyophilized, **B** = Ball milled, **I** = Sieve milled, **M** = Mortar and pestle.
2) Red = ball mill, green = sieve, blue = mortar and pestle.
3) ID = Identification, CFU = Colony forming unit, AVG = Average.
* Clumping refers to the excessive formation of aggregates of the spores within the suspension.

NOTE: These data are for **comparison** of cell count versus CFU/mL **only,** not for absolute CFU/g values of powdered preparations.

Statistical Analysis

Tables 8, 9 and 10 represent the results of the various analyses preformed. Interpretation of the data is exemplified in the following example. For the comparison of drying methods, lyophilized drying and oven drying statistically show indistinguishable effects between them for CFU per gram (Table 8). This is shown visually as a vertical line in the "Differences" column overlapping both rows of method names. Speed Vac drying shares an overlapping line with oven drying indicating the two are statistically indistinguishable. However, Speed Vac drying does not share a line with lyophilized drying indicating that there is a significant difference between the processes in terms of CFU per gram. In summary, the method sets that share one line in the "Differences" column have no significant difference among the methods.

The effect on the number of CFUs per gram (Table 8) was not shown to be significant between the two media types or the two wash methods. There was a significant difference, however, between the lyophilization and Speed Vac drying. There was also a significant difference shown between ball milling and either mortar/pestle or sieve milling.

For effect on particle sizing (Table 9), the medium and wash methods showed no significant difference. Each drying method showed a significant difference compared with the other two. The ball milling also showed a significant difference compared with the mortar/pestle and the sieving methods.

In terms of percent viability of the final product (Table 10), there was no significant difference between the two media or between the two wash methods. The lyophilized method yielded less than half the percent viability compared with the oven dried material and resulted in a significant difference between the two. The difference between the lyophilized product and that of the Speed Vac dried was not shown to be significant, nor was that between the Speed Vac and the oven dried processes in terms of percent viability.

Table 8. Comparison of Variable Methods on Colony Forming Units (CFU) per Gram of Dried Materials.

Variable	Method	Geometric Mean (CFU[a]/g)	Differences (α=0.05)[b]
Medium	Sheep Blood Agar	8.47E+09	
	New Sporulation Medium	1.13E+10	
Wash	Dugway Wash Method	7.67E+09	
	Patrick Wash Method	1.25E+10	
Dry	Lyophilized	5.19E+09	
	Oven Dried	1.16E+10	
	Speed Vac Dried	1.56E+10	
Mill	Ball Milled	1.91E+08	
	Mortar and Pestle Only	6.85E+10	
	Sieve Milled	7.20E+10	

[a]CFU = colony forming unit.

[b]All methods sharing the same vertical line (in the "Differences" column) have statistically indistinguishable effects (Tukey test, α=0.05) on CFU per gram. Shading is for illustration.

Table 9. Particle Diameter Data.

Variable	Method	Geometric Mean (μm)	Differences (α=0.05)[a]
Medium	New Sporulation Medium	3.98	
	Sheep Blood Agar	4.59	
Wash	Patrick Wash Method	4.17	
	Dugway Wash Method	4.37	
Dry	Lyophilized	2.81	
	Speed Vac Dried	4.02	
	Oven Dried	6.90	
Mill	Mortar and Pestle Only	3.48	
	Sieve Milled	3.87	
	Ball Milled	5.79	

[a]All methods sharing the same vertical line (in the "Differences" column) have statistically indistinguishable effects (Tukey test, α=0.05) on particle mean diameter.

Table 10. Particle Viability Data.

Variable	Method	Geometric Mean (%)	Differences (α=0.05)[a]
Medium	Sheep Blood Agar	18.2	
	New Sporulation Medium	18.5	
Wash	Dugway Wash Method	13.7	
	Patrick Wash Method	24.5	
Dry	Lyophilized	10.7	
	Speed Vac Dried	21.2	
	Oven Dried	26.8	
Mill	Mortar and Pestle Only	17.2	
	Sieve Milled	19.5	
	Ball Milled	No data	No data

[a]All methods sharing the same vertical line (in the "Differences" column) have statistically indistinguishable effects (Tukey test, α=0.05) on percent viability.

NOTE: The effects of the two wash methods were almost significantly different (p-value = 0.055).

ANALYSIS

The overall purpose of this work was to provide multiple preparations of dry *B. anthracis* spores in order to determine how the different preparation methods affected the final product. All preparations were derived from two starting paste materials. The subsequent analysis of the preparations by others would, hopefully, provide some insight as to how the spores in the anthrax letters had been prepared. The variables were growth media, spore washing (cleaning) process, drying method, and milling method. It was evident at the start from the macroscopic appearance of the preparations, that there would be differences in the products. This was particularly so when considering the preparations at the end of the drying stage.

The following paragraphs will review the variables of agar type, washing method, drying method, and milling method with some commentary on what impressions the author had in relation to the differences seen. Some general observations will also be made.

The laboratory protocol followed yielded only one final product per set of conditions. That is, there is only one sample for each combination of medium and methods used to obtain a final product. It should be noted that the validity of the statistical analysis is not dependent on the sample size used. The fact that there is only one representative for each unique combination of medium/wash/dry/mill variable does not invalidate the analysis. Additionally, the data were subjected to a logarithmic transformation, which "softens" the effect of outliers that are clearly outside the majority of observations. In short, when data are shown to be statistically significant under the parameters used, then one may feel confident that there really is a difference.

The drawback of the small sample size is that it becomes difficult to statistically determine significant differences at the 95% confidence level. For example, we do not observe any statistical difference between the two wash methods in the analysis. This may reflect that there really is no difference or that we may simply not have enough data points to show that there is one, meaning that the sensitivity is somewhat diminished. If we set the confidence level at 90% or 85%, then a significant difference would have been shown with these same data. That said, even at the 95% confidence level there were some observed differences based on the method of drying, for example, which were enough to say that they were significant and not due to random error alone.

Agar Media

The growth of the Ames strain of *B. anthracis* on the two different agar media manifested differences in the appearance and consistency of the paste material. The paste from the NSM colonial growth was of a lighter color, and what color was present seemed to wash out more readily during the washing steps, leaving a virtually white spore suspension. The material from the blood agar took on the color of the blood, yielding a darker product that did not wash out readily. However, the appearance of the spores microscopically (normal bright field and phase contrast) did not appear different. While some preparations grown on blood agar resulted in higher counts per gram of final dried material compared with NSM agar (e.g., SDLB vs. NDLB and SDLM vs. NDLM), others showed higher counts from material grown on the NSM (e.g., NDVB vs. SDVB, and NDOI vs. SDOI) (Table 6).

The type of agar used is not expected to play a major role in determining the final concentration of spores per gram of dried material if the growth resulted in virtually complete sporulation. If paste contains virtually all spores, then one paste is pretty much equal to another. The amount of the paste per plate may differ. At the conclusion of the growth phase on the agar plates, there was very little else but intact spores and some cell debris in the resulting paste. Any remaining vegetative cells in the paste would naturally have the effect of lowering the spore/gram of paste count by taking up room. Normally during the wash phase, however, any vegetative cells would have been eliminated because they are less dense than the spores and would remain mostly on the surface of the pellet and be washed off prior to resuspensions of the pellet.

The statistical analysis bears out the expectation of the medium making little difference in terms of spore count per gram of finished product. In fact, the choice of media showed no significant difference in either of the other two measuring parameters (particle size and percent viability). The appearance of the final dried material, however, did seem to be affected by the medium of growth. The materials grown on SBA, generally did appear to be darker than that grown on NSM.

Washing Method

The difference between the Patrick and Dugway methods of washing the spores appeared to be considerable at first glance in terms of CFU per gram and particle size. Statistically, however, washing methods were shown to be not significantly different in terms of final, dry product (at the 95% confidence level). While the Dugway method simply resuspended the paste material in cold water by hand with a pipette, the Patrick method added detergent to the cold PBS/spore suspension and homogenized it for several minutes. The addition of the detergent theoretically had the benefit of keeping the spores from forming aggregates to a greater degree than did water alone. Detergent is commonly used for such purposes. This aspect of the difference between the two washing methods leads one to expect higher CFU per gram counts in the Patrick method washed samples (all other things being equal). This expectation was not borne out in the data analysis.

The most notable difference in CFU per gram counts where washing was the only difference in how the preparations were made was in NDLB, where these counts were over 160 times lower than those for NPLB (Table 6). It should be noted that the NDLB sample represents the lowest CFU per gram observation out of the entire 36 final preparations and may represent an anomaly, particularly since the counts per plate were in the low range. All of the ball-milled products were of lower CFU per gram, and because both of these samples were of the ball-milled variety, it may well be that the difference between them is simply exacerbated by having been milled using the ball mill. The difference in CFU per milliliter counts between the Dugway and Patrick washing methods was not nearly as great as the collective comparison as borne out in the statistical analysis.

Drying Methods

The three drying procedures used in the project showed three distinctly different products in terms of macroscopic appearance and consistency. The low technology method of oven drying

is easily performed, whereas the other two methods are more sophisticated and require expensive equipment. Such items may be found used, however, and crude lyophilization can be performed with make-shift setups.

The **oven-dried** material was dark, dense, and very difficult to mill into a powdered form. Upon grinding with an agate or porcelain mortar and pestle, the rock-hard material jumped out of the mortar as it broke into pieces during the grinding. Flattening out the paste more as it dried might have potentially circumvented much of this difficulty. As it was, the paste was simply placed in the oven as a clump and left until dry. Though it was the simplest of the three methods, it yielded the least manageable product.

Oven drying did not necessarily diminish the overall CFU per gram of material, as evidenced by the plate count data (Table 6). The counts were comparable to the counts of materials dried by the other methods. Nor did the method hinder the ability to achieve an appropriate particle size. Though the range of particle sizes was broader in some of the oven-dried preparations and the mean particle size was the largest of the methods over all, it did not preclude obtaining smaller particle sizes of 3 to 5 μm (Table 5). It is noted in the data, however, that the mean particle size was well over 2 μm larger than when using the Speed Vac method and over 4 μm larger compared with the lyophilized method. An unexpected finding was that the oven-dried materials resulted in the highest percent viability when compared with any other preparation variable or method. This may be a by-product of spores on the inner regions of clumps being protected from inactivating "influence", where the more dispersed products were more at risk.

The **Speed Vac**-dried material had a much lighter consistency to it than the oven-dried material. It was also, however, somewhat "spongy" and more difficult to get into a freely flowing state. For example, when the mortar and pestle were used to "mill" the Speed Vac-dried materials, the powders seemed to mat together rather than break apart into smaller particles. The statistical analysis showed that, in terms of CFU per milliliter, the Speed Vac method was not significantly different than the ball mill method but was different when compared with the lyophilization method, resulting in significantly more CFU per gram, even though the mean particle size was also significantly larger (nearly by 1.5 times). This is at least partially explained by the percent viability of Speed Vac-dried materials being approximately twice that of the lyophilized preparations, thongh this was not shown to be a significant difference.

The **lyophilized** material was always more crystalline than the other preparations, regardless of other parameters (i.e., agar type, washing method, or milling method). Generally, the lyophilized materials yielded products that were of smaller particle sizes. It was expected that the lyophilized materials would yield higher CFU per gram due to the comparatively gentler process entailed, at least compared with the oven-dried material. However, the concentrations of oven-dried material, though not shown to be significantly different statistically, were approximately twice that of the lyophilized material.

Note that the lyophilized preparations NDLI and NDLM showed 0 and 1% viability, respectively (Table 7). These are the only observations with less than 9% viability. Most of the 36 preparations were in the range of 20 to 39%.

Milling Method

The methods used for milling were all relatively simple. Each method provided preparations that resulted in mean particle sizes that were very suitable for a respiratory threat. None of these preparations had a fluidizer added to them. The effect of a fluidizer (e.g., silica) would have yielded particles that would resist any clumping tendencies and therefore would likely have had smaller mean particle sizes particularly for the preparations from the ball mill. However, even without this aid, the particle sizes in most preparations had means of less than 8 μm using the aerosol generation and sizing methods described herein, and many were in the 2 to 3 μm range (Table 5).

The **ball mill** yielded material that, in all cases but three (NPVB, NDVB and NPLB), had CFU counts per gram of material that were much lower compared with the other milling methods (Table 6). In the light microscope photographs of the preparations, each of the ball-milled samples also showed a high degree of clumping in spite of the samples being homogenized in liquid suspension during the sample preparation for assay and microscopy work. This suggests that either the ball mill procedure used in this work inactivated the spores, or the spores were viable, but counts were diminished by having the colony forming units 10 to 100 times larger than those in the other preparations. In light of the clumping, the latter suggestion seems more likely.

The aerosol risk using this method is low if the gasket on the milling jar is well seated. Also, very little attention to the process is required once the jar is set in motion. When the jar is opened, however, a clear risk is present.

The **sieve method** is what has been used at DPG to mill *B. anthracis* over the past several years (even prior to the anthrax letters). Early on, these sieves were "homemade" using fine mesh screens and gluing them into caps of 90-mL tubes that had the centers cut out. The ball bearings were then manually agitated by shaking to force the powder through the screens. These sieves were stainless steel, and the sieving action with the ball bearings was driven by an air piston mounted on the outside of the container. Both methods have yielded very good results in terms of getting respirable particle sizes.

The aerosol risk using this method is very low because the containers are air tight. It is clear, however, that once the lid is removed that aerosolization is a predetermined certainty.

The **mortar and pestle** method is arguably the lowest technological method used of the three, and yet it is the fastest. It is evident from the sizing data that even this "low tech" method provided significant quantities of viable particles of less than 10 μm suitable for aerosolization and lung deposition.

Mortar and pestle units are available on the open market and are commonly sold for household kitchen use. Unless contained within a glove box of some sort, the process will result in a contaminated environment and a hazard to the unprotected operator.

Elemental Analysis

The osmium detected in the samples may be accounted for by the OsO_4 procedure used to sterilize the preparations; therefore, a standard was used to try and subtract out any background. The color-mapping of osmium and antimony on the image overlap each other significantly and suggests that the antimony may be a false identification (had they been separated in the pictures, then the assumption would be that both were present). As it is, there are no clear-cut data to suggest the presence of antimony.

General Comments About the Process

The principal author has been culturing *Bacillus anthracis* for over 20 years using various strains, media, and equipment. Drying the spores has been a relatively new activity (since 1999). Most procedures used in the production of the dried preparations were not technologically sophisticated. The growth on agar plates may readily be performed on a low budget with minimal equipment and a high expectation of pure spore product. The growth medium required is not exotic and could be obtained in virtually any microbiology laboratory and sterilized in a home pressure cooker in commonplace containers such as reusable mason jars. Petri dishes are not required nor are other laboratory supplies. A makeshift incubator may be as simple as an ice chest-type container sitting on a heating pad.

Harvesting to obtain the spores in a consolidated mass of paste may be easily done with a clean rubber spatula. At this point, the aerosol risk is low and the danger of bacterial contamination is not an issue, as the concentration of the spores is very high and there is little risk of losing the product due to growth of a contaminant prior to drying (if done reasonably soon).

Once harvested, it may or may not be necessary to wash the material for preparation of a "weaponized" material (all materials in this study were washed.). The washing of spore paste was done quickly in this study using a centrifuge. It could be done by allowing the spores to naturally settle out of suspension. This takes considerably more time (perhaps a week for a 1-L suspension to get 90% reduction in volume), especially if there was more than one wash step, and may enhance the quality of the product. The last settling step would take longer because it would be desirable to remove as much liquid as possible to make the paste easier to dry.

As shown by this study, the drying of a spore paste can be done effectively in several ways. Lyophilization and Speed Vac drying are on the high end of the technology scale and apparently do not give so high a return on the investment as to overlook oven drying. The positive aspect of drying by lyophilization or Speed Vac is that the containers are enclosed within the drying equipment and the final products are more readily milled. The oven-dried material obtained in this study was very rigid. This made milling more difficult, messy, and potentially more dangerous to the operator.

Silica is widely available on the open market and used in many products. It was not used in any of these preparations. Milling of dried material without silica added to the product is far less risky than with silica, based on previous observations with siliconized spore powders. Nevertheless, even without silica, one is at significant risk of exposure when milling dried *B. anthracis*

spores without appropriate protective equipment. In this regard there is no more important protection required than that for the respiratory tract. This may be provided with commercially-available High Efficiency Particulate Air (filter) (HEPA)-filtered respirators. To protect the work environment (and provide additional protection for personnel), an airtight glove box of some sort with a HEPA-filtered outlet would be essential. These two items, along with a healthy understanding of the inherent danger, would eliminate the majority of risk associated with the drying and milling processes. A suitable glove box may be purchased ready to use. There are even disposable glove bags that would provide suitable protection. Alternatively, one may modify existing commercial off-the-shelf (COTS) products (e.g., a spark plug sand-blasting box) or dispense with containment altogether and do the procedures with a mask only and out in the desert.

A prime difficulty in pursuing the production of spores is obtaining a starter culture. It is common knowledge that there are places in the world where animals become infected and die of anthrax. However, finding and isolating the organism from the environment is not a trivial accomplishment. An easier source would be a laboratory that works with known infectious strains. After having obtained a strain of the organism from the environment or a laboratory, it would still be necessary to demonstrate its virulence against humans. It should be noted that it would be virtually impossible to prevent someone working with such a strain in a laboratory from smuggling a starter culture out.

The author saw the material from the Senator Leahy letter firsthand after it was transferred to a glass (or plastic) tube. It was very free-flowing and appeared to aerosolize readily. The material produced in the present project also had the quality of being easily aerosolized and the particle size from each preparation was easily within the range suitable for inhalational infection.

The crude material from the Washington Post letter, however, was of very poor quality almost like brown sugar crystals. It had the appearance of paste material that had been air dried, perhaps in an oven without further processing. It certainly was not of a quality that would be considered "weaponized" and would not present a readily aerosolizable product.

Finally, excluding the difficulty in obtaining a starter culture, it is the author's professional opinion that the level of product quality achieved in this project could readily be attained without a large physical footprint, a large outlay of money, or an excessive risk to a knowledgeable operator. Based on the CFU per gram and particle sizing data, virtually every combination of methods used herein resulted in a product suitable for the assumed purpose of the person(s) who sent the anthrax letters. Presumably that purpose was to cause panic and terror by effectively using a lethal agent that was aerosolizable from an envelope. It is hoped that the preparations produced and provided will allow analysts to give some insight as to how the materials in the letters were made.

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APPENDICES

Appendix A. Death Certificates

CSTE-DTC-DP-WD-L 4 October 2004

MEMORANDUM FOR RECORD

SUBJECT: Certificate of Non-viability

1. The following has been rendered non-viable (killed or inactivated):

a. Name of organisms(s): *Bacillus anthracis* Ames: various aliquots from pooled batches of 07JAN03, 23AUG03, and 28AUG03 and designated Acetone dried NSM/Patrick or SBA/Patrick (meaning New sporulation medium or Sheep blood agar with Bill Patrick's washing steps.). Additionally, each preparation was either allowed to dry for an additional 7 days or not. This was also labeled on the vials.

b. Place and date of sterilization: Bldg. 2032, U.S. Army Dugway Proving Ground, UT, Dosage of 3.9×10^6 Rads on 16 SEP 2004.

2. The organism listed in paragraph 1.a. was **confirmed** non-viable (killed or inactivated) by the following:

a. Procedure used: Approximately 10% of each pre-inactivated *B. anthracis* powdered product was suspended in sterile Tryptic Soy Broth (TSB). An uninoculated tube of broth was the negative control. An additional tube was inoculated with *B. anthracis*, Ames as a positive control. All were incubated at 37°C for 72 hours. The positive control showed active growth of culture within 24 hours. The TSI broth with inactivated product and the negative control showed no growth. Additionally, 0.5 mL from each broth culture was placed on blood agar and incubated for 36 hours at 37°C to verify no growth. Results were same as for the broth.

b. References (Article, SOP, etc.): "Inactivation of Biological Agents and Simulants for Antigen Production", WDL-BIO-147, rev 0. Batch record 5.

c. Place and date of confirmation: Life Sciences Test Facility, Life Sciences Division, U.S. Army Dugway Proving Ground, UT. 20-27 SEP 2004.

3. Based on our knowledge of the sterilization procedure used in paragraph 1 and based on the results of the confirmation procedure used in paragraph 2, we certify, to the best of our knowledge, that the above listed organisms are non-viable.

/signed/
DANIEL D. MARTIN
Project Officer

[redacted]
Life Sciences Division

[redacted]
Biosafety Officer
Life Sciences Division

DTC0121

CSTE-DTC-DP-WD-L

9 September 2004

MEMORANDUM FOR RECORD

SUBJECT: Certificate of Non-viability

1. The following has been rendered non-viable (killed or inactivated):

a. Name of organisms(s): *Bacillus anthracis* Ames: lot number 20MAY04.

b. Place and date of sterilization: Bldg. 2032, U.S. Army Dugway Proving Ground, UT, Dosage of 3.5 x 10^6 Rads on 19 AUG 2004.

2. The organism listed in paragraph 1.a. was **confirmed** non-viable (killed or inactivated) by the following:

a. Procedure used: A 1.3 g of the pre-inactivated *B. anthracis* paste product was suspended in sterile Brain Heart Infusion (BHI) broth. An uninoculated tube of broth was the negative control. An additional tube was inoculated with *B. anthracis*, Ames as a positive control. All were incubated at 37°C for 72 hours. The positive control showed active growth of culture. The BHI broth with inactivated product and the negative control were clear showing no growth.

b. References (Article, SOP, etc.): "Inactivation of Biological Agents and Simulants for Antigen Production", WDL-BIO-147, rev 0. Batch record 5.

c. Place and date of confirmation: Life Sciences Test Facility, Life Sciences Division, U.S. Army Dugway Proving Ground, UT. 12Jul04 through 16Jul04

3. Based on our knowledge of the sterilization procedure used in paragraph 1 and based on the results of the confirmation procedure used in paragraph 2, we certify, to the best of our knowledge, that the above listed organisms are non-viable.

/signed/
DANIEL D. MARTIN
Project Officer

[redacted]
[redacted] Life Sciences Division

[redacted]
Biosafety Officer
Life Sciences Division

b6
b7C

Appendix B. Test Execution Directive

DEPARTMENT OF THE ARMY
HEADQUARTERS, U.S. ARMY DEVELOPMENTAL TEST COMMAND
314 LONGS CORNER ROAD
ABERDEEN PROVING GROUND MD 21005-5055

REPLY TO ATTENTION OF

CSTE-DTC-TT-S (70-10p)

S: 28 Mar 02
7 MAR 2002

MEMORANDUM FOR U.S. Army Dugway Proving Ground, West Desert Test Center (CSTE-DTC-DP-WD-A), Dugway, UT 84022-5000

SUBJECT: Test Execution Directive: Analytical Chemistry Analyses of Anthrax Powders, DTC Project No. 8-CO-480-000-006

1. Reference, Letter, U.S. Department of Justice, Federal Bureau of Investigation, 12 Dec 01, subject as above (enclosure 1).

2. Subject testing is assigned to the U.S. Army Dugway Proving Ground (DPG) for accomplishment in accordance with reference 1. DPG is authorized to begin test planning upon receipt of funds.

3. Direct communication with the test sponsor is authorized concerning details.

4. Special Instructions:

a. DTC Project No. 8-CO-480-000-006 is assigned.

b. DPG is hereby directed to perform the analytical chemistry analyses of anthrax powders. An outlined preparation procedures for Bacillus globigi is provided in enclosure 2. DPG test director is required to contact Supervisory Special Agent (SSA) [redacted] to discuss the FBI's needs in detail.

c. Abbreviated test plan and a test report are required. Two copies of the abbreviated test plan and test reports will be provided to this headquarters (ATTN: CSTE-DTC-TT-S) and DOJ, FBI, 601 4th Street, NW, Washington, DC 20535.

d. DPG will submit a cost estimate indicating reimbursable funds needed for the agent test to DOJ FBI (SSA [redacted], with a copy furnish to this headquarters, (CSTE-DTC-TT-S [redacted], NLT 28 Mar 02.

e. Points of contact are:

(1) DOJ FBI: SSA [redacted]

(2) DTC: [redacted] CSTE-DTC-TT-S, DSN 298-1329.

5. Upon receipt of this directive, immediately review test milestone schedule in light of known other workload and projected available resources, in accordance with DTCR 73-3. If rescheduling is necessary and the sponsor nonconcurs, forward a memorandum citing particulars, together with recommendations, to DTC, (CSTE-DTC-TT-S [redacted] with an information copy to CSTE-DTC-TT-B within 15 days after receipt of this directive. Reschedules

concurred in by the sponsor can be entered directly by your installation/test activity by the above date.

6. The analyses of anthrax powders will be conducted in the Life Science Test Facility (LSTF). Safety precautions should be observed throughout the test. Laboratory SOP will be followed.

7. Environmental documentation prepared by test sponsor to support this test would not contribute to the environmental analysis to be conducted by the test center; therefore, environmental documentation has not been requested. Site specific environmental documentation must be prepared and coordinated with the DPG EQC prior to test initiation.

8. Security: Subject test is unclassified.

9. The analyses of anthrax powders shall be performed such that energy consumption and conservation are considered throughout the study.

FOR THE COMMANDER:

2 Encls

[redacted]
C3 and IEW Division
Directorate for Test and Technology

Appendix C. Photographs

Figure C.1. Incubation of Inoculated Blood Agar Plates.

Figure C 2. Harvesting Spore Paste From Blood Agar Plates.

Figure C.3. Closeup of Paste Harvest.

Figure C.4. Depositing Paste Within Sterile 90-mL Tube.

Figure C.5. Depositing Paste in Container. Note That Edges Get Dry and Flake Off.

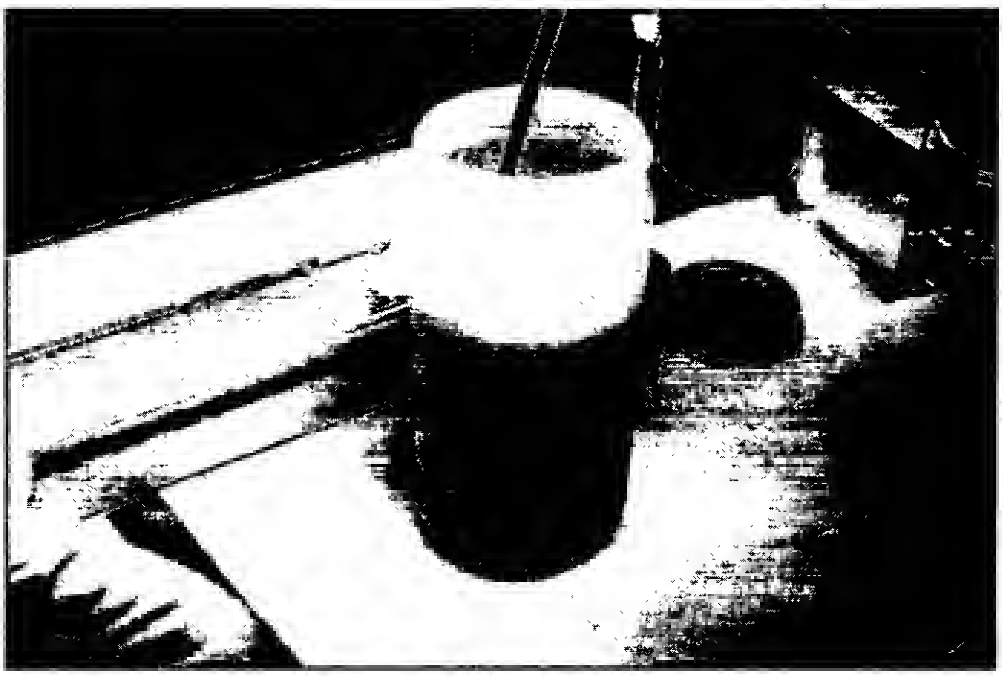

Figure C.6. Paste From Harvest Resuspended in Wash Fluid.

Figure C.7. Resuspension Transferred to Blender Jar.

Figure C.8. Placing Bleach-Soaked Towel Over Lid Prior to Blending.

Figure C.9. After Blending. Note "Head" of Foam That is Rich in Spores.

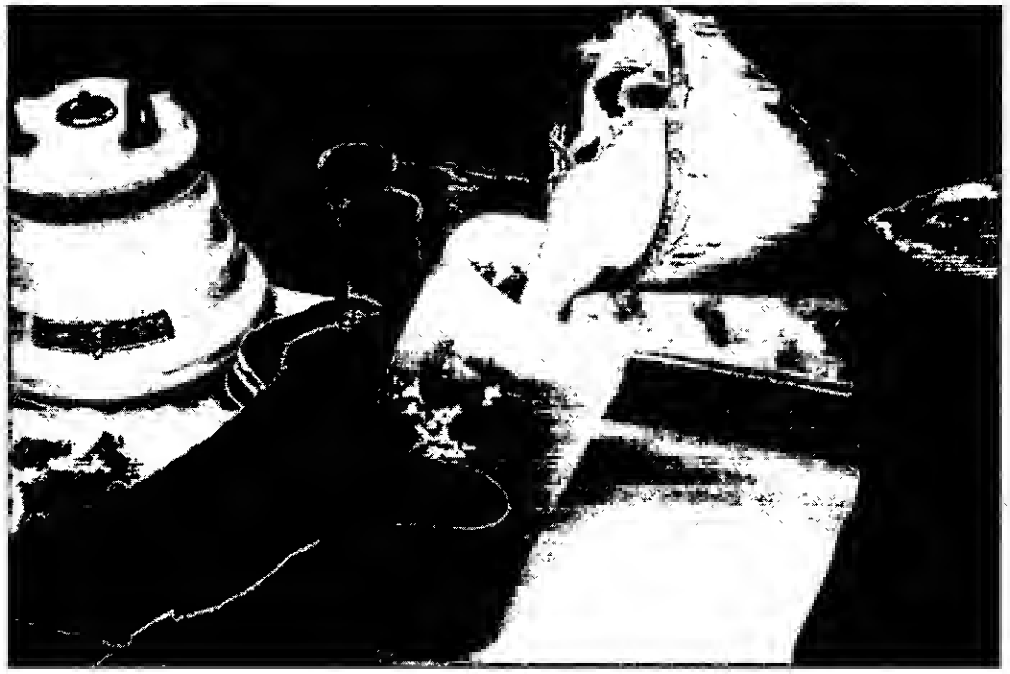

Figure C.10. Transferring Blended Resuspension to Centrifuge Bottle.

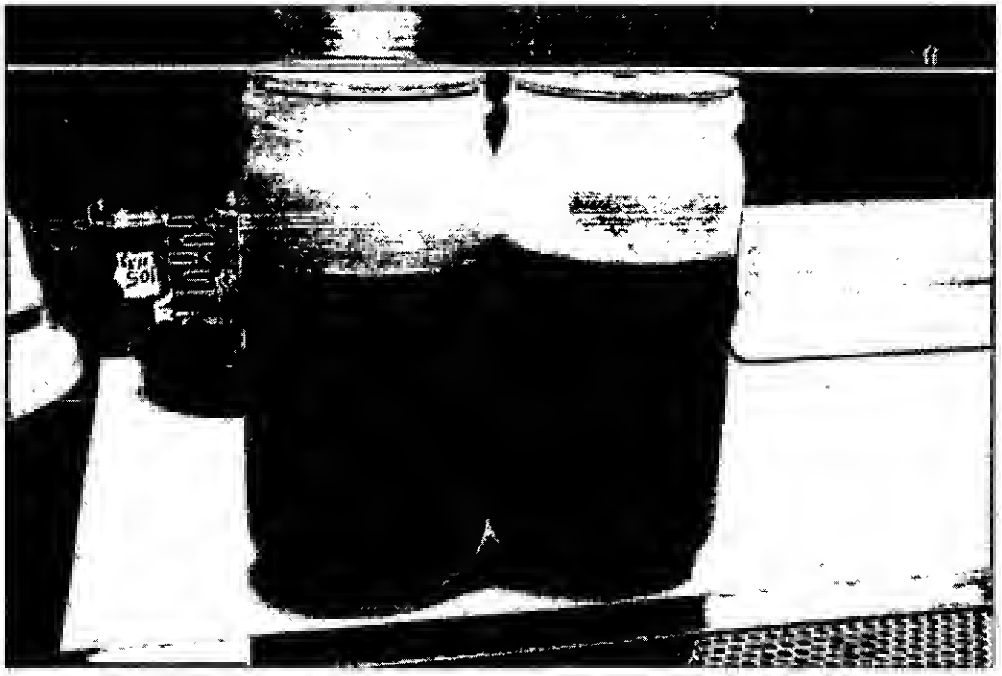

Figure C.11. After Centrifugation. Note Large Pellets of Spores.

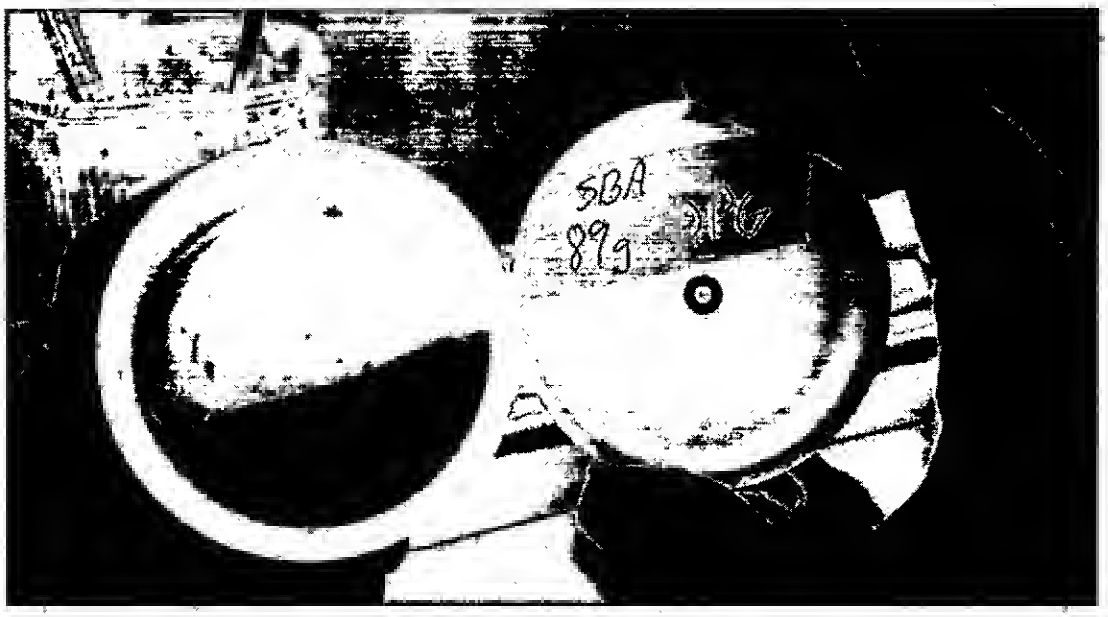

Figure C.12. Pellet From Wash. Note Size of 89-g Pellet.

Figure C.13. Aliquot in Drying Oven After Washing.

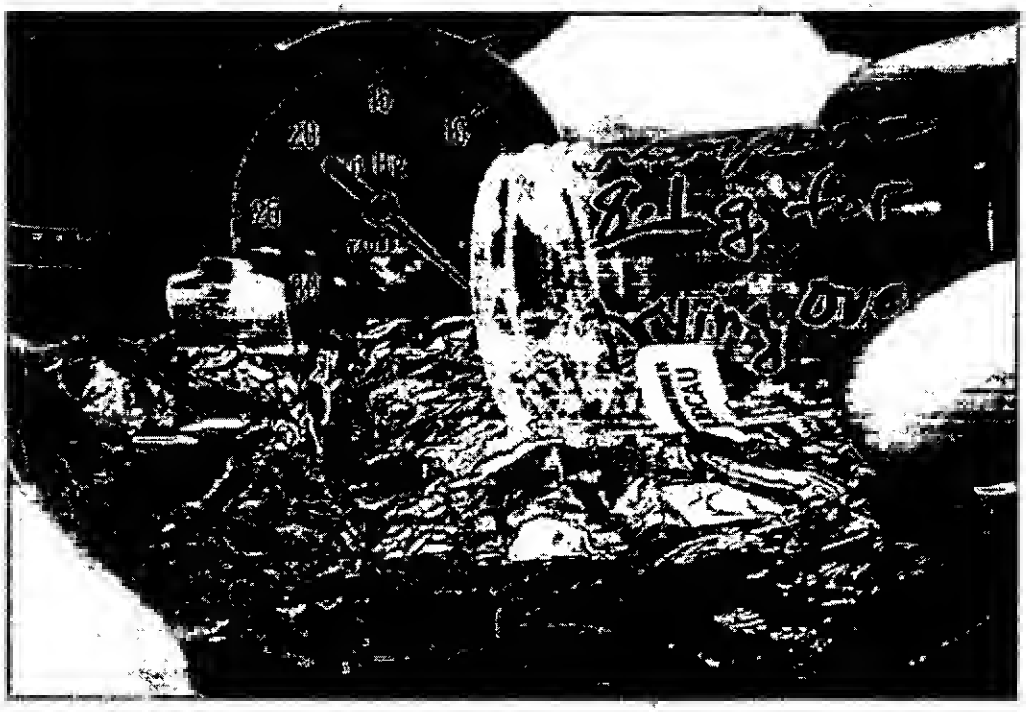

Figure C.14. Flakes of Dried Oven-Paste.

Figure C.15. Grinding Oven-Dried Paste With Mortar and Pestle.

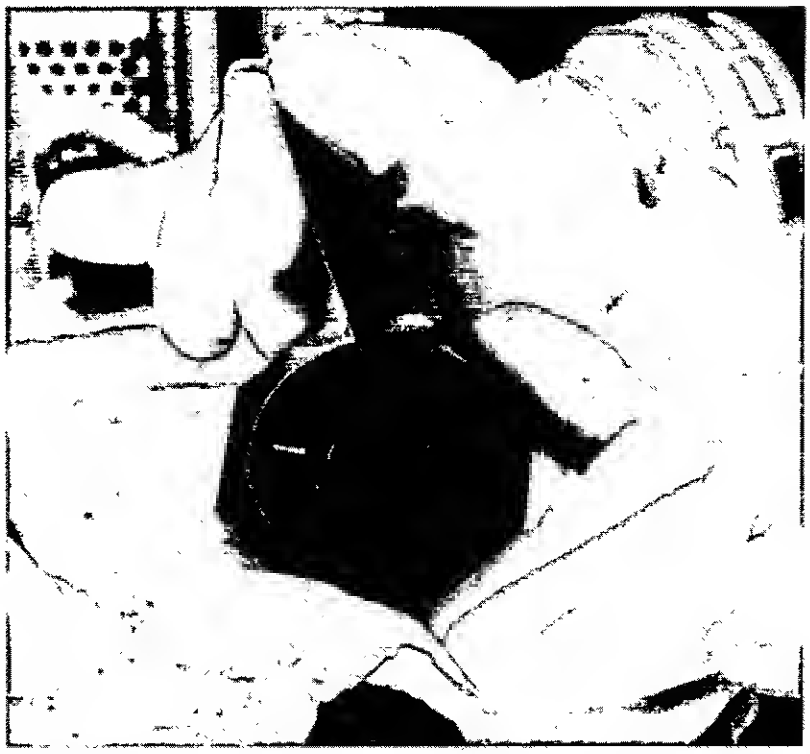

Figure C.16. Grinding Powdered Spores With Mortar and Pestle.

Figure C.17. Transfer of Ground Powder to I-Chem™ Jar.

Figure C.18. I-Chem™ Vial Used for Storage of Final Preparation.

Figure C.19. Another Example of Oven-Dried Spore Paste.

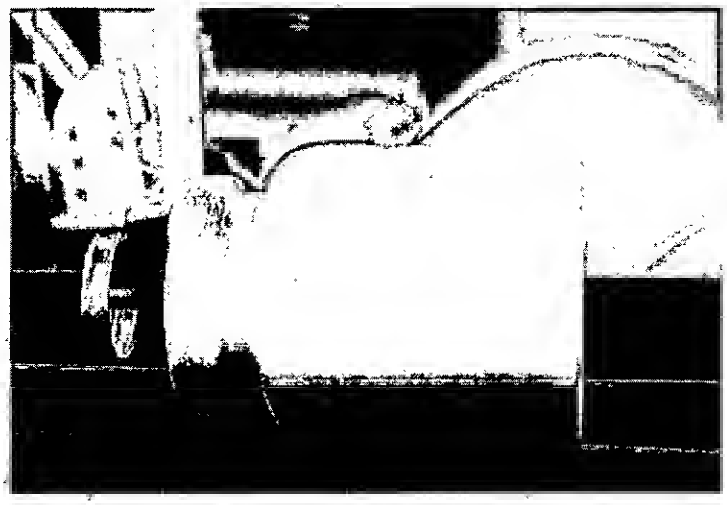

Figure C.20. Milling Procedure in Glove Box.

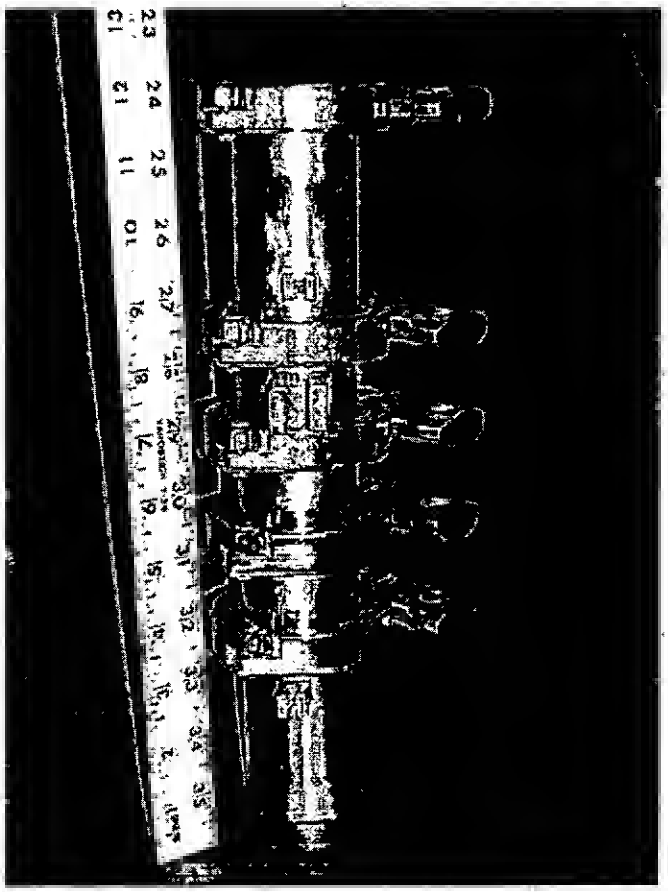

Figure C.21. Assembled Sieve Mill Apparatus Without Air Piston.

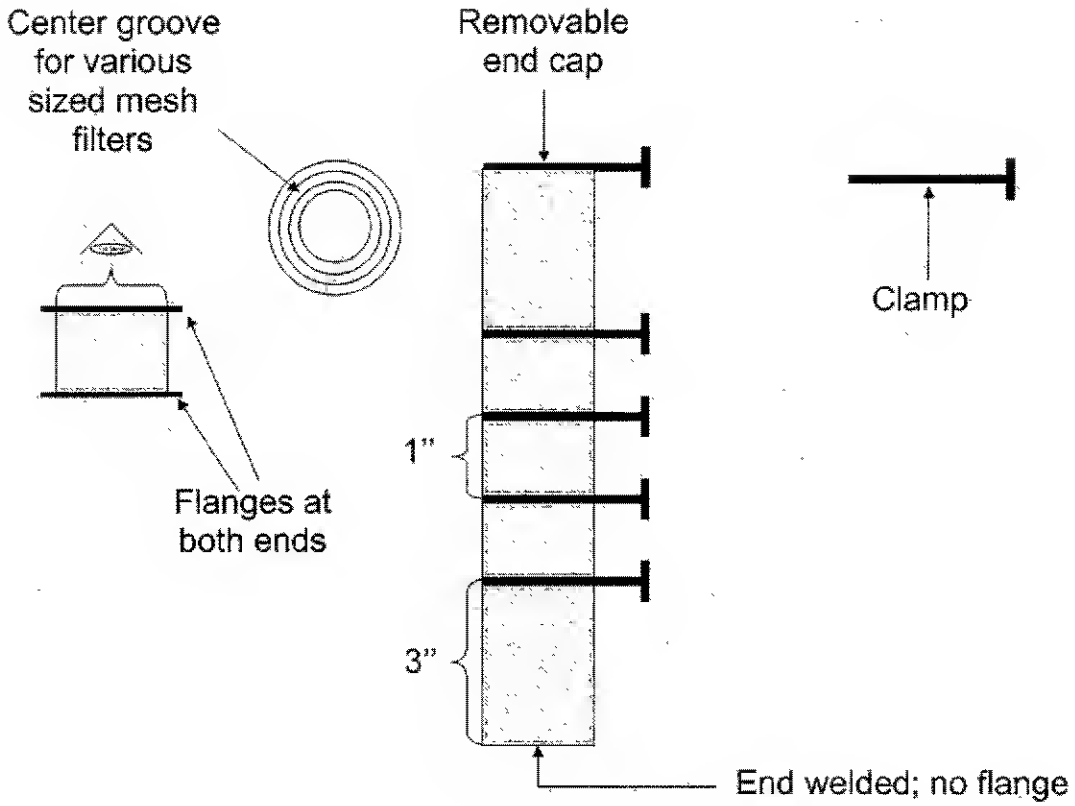

Figure C.22. Drawing of Sieve Mill.

Appendix D. National Environmental Policy Act (NEPA) Documentation

REQUEST FOR
NATIONAL ENVIRONMENTAL POLICY ACT DOCUMENTATION
(NEPA)

The following information is required for each test or other action, including training. Submit the information to WD-ET (Environmental Technology Office) for preparation of NEPA documents. Point of contact is [redacted] at extension [redacted] or [redacted] at [redacted] Submission of the file as a computer file is preferred and will save time. Include a copy of the test plan, safety assessment, human use committee review, and test directive. Enter a summary in the form, do not just reference the test plan or other document.

1. TRMS TITLE: Analytical Chemistry Analysis of Anthrax

DATE NEEDED: 31 OCT 2002 DATE REQUESTED: 17 OCT 2002

TRMS Number: 8-CO-480-000-006(K) JONO: 2D0Y06

200: _____ (Env. Doc. Rcvd.) 210: _____ (Site-specific Env. Doc.)

220: _____ (No Env. Doc. Rqd.) 440: _____

Classification: Unclas. TD initial: _____

Security Classification Guide: attach copy

2. Test Director: Daniel D. Martin PHONE: 3020

3. DESCRIPTION OF PROPOSED ACTION

a. Background: The FBI has requested that DPG provide them with some dried and milled preparations of *Bacillus* species (including *anthracis*). These materials will be grown on agar plates and in broth principally in the BL-3 suite of the LSTF. Upon completion of the project (or as each phase is completed), the Bureau will provide arrangements to transport the material to their facilities.

b. Test Item (description): NOT A TEST

c. Objectives: Produce approximately 50-100 g of dried, milled powder of *Bacillus anthracis*, *cereus*, and other species. Also provide samples of each intermediate from start to finish including all reagents used in the process.

d. Test Conduct: All activities with pathogenic strains will take place in the BL-3 containment areas of the LSTF. Each species will be grown either in broth or agar and the spores harvested. The resulting paste will be resuspended in water and "washed" several time by centrifugation and repeated resuspensions. The paste material will then be dried via natural drying, solvent wash, and lyophilization. The resulting product will be milled by sieve action, ball milling, and mortar and pestle methods. The high aerosol risk activities will take place within a class 3 glove box. The final products will be characterized by electron microscopy, particle sizing, plate count, gram stain, spore stain, etc.

e. Materials and equipment: Sheep blood, killed *Bacillus anthracis, Bacillus thuringiensis, Bacillus cereus,* high speed centrifuge, broth in fermentor, incubator, blood agar in Petri dishes, glove box, biological safety cabinet, blender, ball mill, electron microscope, aerosol particle sizer.

f. Quantities: (This is not restricted to quantities of materials released to the atmosphere. For example, include the mount of obscurant per grenade. If the material to be released is burned or otherwise modified, we must have details on the combustion products.)

50-100 g of dry, powdered *Bacillus anthracis* material; approximately 20 g of *B cereus* and *B. thuringiensis*. Quantities to be aerosolized for analysis of particle size will be less than 1 gram.

g. Location of test: LSTF (bldg 2029) rooms: multiple laboratory rooms throughout the building.

h. Excavation Permit: NA

Furnish drawing/map of the area if construction is required.

i. Hazard Assessment, Safety Release, or Plan: (Attach a copy) in preparation

j. Disposal of test items, include damaged items, nonhazardous waste, and hazardous waste: All hazardous waste will be autoclaved out of the BL-3 areas.

4. ANTICIPATED DATES AND DURATION OF PROPOSED ACTION:
1 November 2002 through 30 September 2003

5. AVAILABLE NEPA DOCUMENTATION: (Attach copies) NA

6. TEST EXECUTION DIRECTIVE: (Attach a copy) Attached

7. COPY OF UNCLASSIFIED TEST OR OPERATIONS PLAN: (Attach a copy) Customer "Test Plan" attached.

8. HUMAN USE COMMITTEE REVIEW: NA

NOTES:

This form is designed to collect information for preparation of a record of environmental consideration (REC). A REC must be based on an environmental assessment (EA) or categorical exclusion. If neither is appropriate or available, and environmental assessment will be required. We will require considerably more information and funding for preparation of an EA.

Usually a REC requires between two weeks and a month for preparation and review for signature. An EA can take six months to complete and requires a 30-day public comment period. Also, and EA could conclude that and environmental impact statement (EIS) is required. Preparation of an EIS may require a year or more.

Therefore, availability of a valid EA that a REC can be tiered from is important. You can save time and money if there is an appropriate and valid EA.

If you cannot fill out the information needed, contact the Environmental Technology Office and we can work on the problem. If you cannot supply the information required for a REC, one cannot be prepared and you cannot run the proposed test. Thus, advance planning is essential.

Appendix E. Hazard Analysis

HAZARD ANALYSIS
Bacillus anthracis Analysis

OFFICE: WD-L-T PREPARED BY: [redacted] DATE: 22 May 2002

This hazard analysis is used to quantify the risks involved in the operation based on the severity and probability of the hazards for the test, as well as the controls implemented to minimize the level of risk. This hazard analysis is developed in accordance with Army Regulation 385-69, Biological Defense Safety Program. The hazard analysis is an integral component of the risk management process because it identifies the risks involved with the test and the controls designed to mitigate these hazards. The test director will ensure these controls are implemented for the duration of the test.

Operation	Culture, concentration and drying of *B. anthracis*
Hazard	Exposure to agent of biological origin (ABO) and or acetone/flammability hazard..
Result	Death or permanent disability
Initial RAC	I-B, Catastrophic – Likely; **EXTREMELY HIGH**
Controls	• Operators will read, understand, and sign appropriate SOP's. Supervisors will ensure operators are trained and certified. Supervisors will ensure that operators wear designated protective equipment. • Concentration and drying will be done in a glovebox in BL-3. • All other work will be done in approved Biosafety cabinets in BL-3. • Limited access to room (project personnel only during operations). .
Final RAC	I-E, Catastrophic – Improbable; **MEDIUM**
Operation	Analyzing ABO samples
Hazard	Spill of agent. Exposure to ABO.
Result	Death or permanent disability
Initial RAC	I-B, Catastrophic – Likely; **EXTREMELY HIGH**
Controls	• All analyses will take place inside a glovebox or certified biosafety cabinet in BL-3, used in accordance with DPG SOPs WDL-300 and 326. • Test participants will utilize appropriate levels of PPE with respect to the agent being analyzed. • Test participants handling agents will be in the Special Immunization Program and be current on anthrax booster. • All test participants will have read SOPs WDL-330, 328, and 326 within the past 6 months.
Final RAC	I-E, Catastrophic – Improbable; **MEDIUM**

Operation	Manipulations using Sharps and Glassware (sidearm glass flask, blender cup rotor/blades)
Hazard	Cuts or puncture wounds due to exposure to sharp instruments or broken glass
Result	Possible bleeding and/or infected wound and or percutaneous exposure to agent.
Initial RAC	II-D, Critical – Seldom; **MEDIUM**
Controls	• Wear appropriate gloves during all procedures with sharps and glassware. Follow the laboratory procedures for sharps use and disposal (SOP-WDL-SAF-326). Have an appropriate sharps container available for disposal of sharps. Also have a broken glass container available. • All test participants will have read SOP WDL-SAF-326 within the past 6 months.
Final RAC	II-E, Critical – Improbable; **LOW**
Operation	Cleanup
Hazard	Exposure to ABO and or acetone (flammability hazard)
Result	Death or System Loss
Initial RAC	I-C, Catastrophic – Occasional; **HIGH**
Controls	• All areas will be decontaminated prior to cleanup. • Appropriate PPE. • Appropriate waste storage for acetone waste including cool ventilated area away from fire hazard, oxidizing material and acids.
Final RAC	I-E, Catastrophic – Improbable; **MEDIUM**
Operation	Handling waste
Hazard	Exposure to ABO and or acetone
Result	Injury or system damage
Initial RAC	II D, Critical – Seldom; **MEDIUM**
Controls	• All waste material will be autoclaved.
Final RAC	II-E, Critical – Improbable; **LOW**

Safety Procedures and Hazard Analysis Approved By:

Original signed *2 December 2002*
[redacted] DATE
Bio Safety Officer

Original signed *2 December 2002*
[redacted] DATE
[redacted] Compliance Office

Appendix F. References

1. Memorandum, U.S. Army Developmental Test Command, Aberdeen Proving Ground (APG), Maryland, 7 March 2002, subject: Test Execution Directive for Analytical Chemistry Analysis Of Anthrax Powders, Test Project No. 8-CO-480-000-006.

2. John J. Friel, *X-Ray and Image Analysis in Electron Microscopy*, Princeton Gamma-Tech, Princeton, New Jersey, 2004.

3. U.S. Army Dugway Proving Ground (DPG), Utah, Abbreviated Test Plan for The Analytical Chemistry Analysis of Anthrax Powders, WDTC-TP-23 JAN 2003.

Appendix G. Abbreviations

ANOVA – analysis of variance
AVG – average
B – ball mill method
BL3 – biosafety, level 3
CFU – colony forming units
COTS – commercial off-the-shelf
D – Dugway wash method
DPG – U.S. Army Dugway Proving Ground
DTC – death certificate
EDS – energy dispersive spectroscopy
FBI – Federal Bureau of Investigation
I – sieve method
ID – identification
L – lyophilized
M – mortar and pestle method
N – new sporulation medium
NMRC – Naval Medical Research Center
NSM – new sporulation medium
O – oven dried
OsO_4 – osmium tetroxide
P – Patrick wash method
PBS – phosphate buffered saline
RH – relative humidity
S – sheep blood agar
SBA – sheep blood agar
TSA – Tryptic Soy Agar
V – Speed Vac dried
WFI – water for irrigation

Appendix H. Distribution List

Addressee	Copies
FBI Washington Washington Metropolitan Field Office ATTN: [redacted] 601 4th Street, N.W. Washington, D.C. 20535-0002	3
FBI Laboratory ATTN: [redacted] (CBSU) 2501 Investigation Parkway Quantico, VA 22135	3
U.S. Army Dugway Proving Ground (CSTE-DTS-DP-WD-SP-M/Technical Library) Dugway, UT 84022-5000	2